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STUDIES IN INFLUENZA AND PNEUMONIA

VI. THE LEUKOCYTIC REACTION IN INFLUENZA AND INFLUENZAL PNEUMONIA

E. C. Rosenow

Division of Experimental Bacteriology, The Mayo Foundation, Rochester, Minnesota.

Leukopenia, or absence of leukocytosis, has come to be regarded as an important aid in the diagnosis of influenza, and a persistent leukopenia as of bad prognostic import. The cause of the leukopenia has been the subject of much speculation. It is thought by many observers that an unknown virus produces the initial leukopenia, and that this virus interferes with the leukocytic response when secondary infection by streptococci or pneumococci is believed to occur as pneumonia develops.

No matter what the cause of influenza may be, the absence of leukocytosis is a striking phenomenon. The exact leukocytic reaction, however, throughout the initial influenzal attack and in influenzal pneumonia in fatal and nonfatal infections has not been determined, nor has a systematic study of the leukocytic reaction in animals been made following injections of the different species of bacteria that are so commonly isolated in influenza. It was therefore thought worth while to determine the leukocytic curves in the spontaneous disease and in experimental animals in which the dosage and type of organism introduced could be accurately controlled and might throw light on the question as to the cause of leukopenia in influenzal infection and determine more accurately the prognostic significance of the lack of leukocytic reaction in this disease.

In figure 1 the curves indicate the average leukocyte counts by days in influenza and influenzal pneumonia in man, and experimental influenzal pneumonia in guinea-pigs, according to fatal and nonfatal infection. There were no deaths from influenza without lung involvement among the cases included in part 1 of figure 1. The dotted line represents the count in the cases in which fatal attacks of influenzal pneumonia later developed. The average count for the first six days in influenza was about 6,500, and was approximately the same in the fatal

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and nonfatal cases. From the seventh to the tenth day the count rose somewhat in the patients who recovered, while it dropped in those who later developed influenzal pneumonia from which they succumbed. It will be noted that, contrary to the general belief, the average leukocyte count when influenzal pneumonia developed was no higher than during the initial influenzal attack. The count in the nonfatal infections then gradually rose, while in the fatal infections it rose for two days and then gradually declined. This finding is in accord with that of Haase and Wohlrabe[1] who also found that there is a tendency of the leukocyte count to rise in the later stages of influenza and influenzal pneumonia.

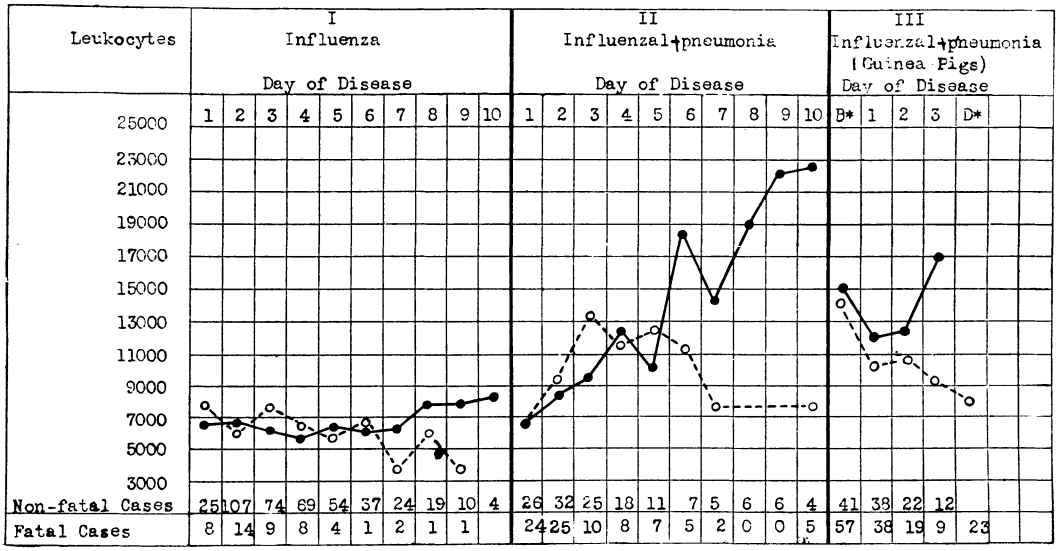

Fig. 1.—Average leukocyte count by days in influenza and influenzal pneumonia in guinea-pigs according to fatal and nonfatal infection. —denotes the average leukocyte count in nonfatal infection; —— average leukocyte count in fatal infection; * B, before injection; * D, after death.

Marked individual differences were noted in the leukocytic response in guinea-pigs following injection of bacteria from patients with influenza. If a series of animals was injected with the same dose of a given strain, nearly all showed a drop in the number of leukocytes; the drop in some was slight, in others it was marked, and occasionally an animal showed leukocytosis. There was no definite relation between the mortality rate and the initial leukopenia, but a persistently low count occurred more often in the fatal infections. In part III of figure 1 the average leukocyte count by days in nonfatal infections shows a decided drop for two days following injection and then a rise, while in the fatal infections there was a progressive diminution. These curves are believed to be accurately representative, since they include, as indicated, the average of a large series of animals and correspond roughly with the curves in the spontaneous disease. The leukocyte count in apparently normal guinea-pigs varies greatly, but counts made repeatedly show that it tends to remain at a given level over a period of some days. The injections were made in various ways with sputum, primary cultures, exudates and pure cultures of green-producing streptococci from the sputum or lungs in undoubted cases of influenzal infection.

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[1] Haase, N., and Wohlrabe: Ueber das Blutbild bei Influenza, Deutsche. med. Wchnschr., 1918, 44, p. 1383.

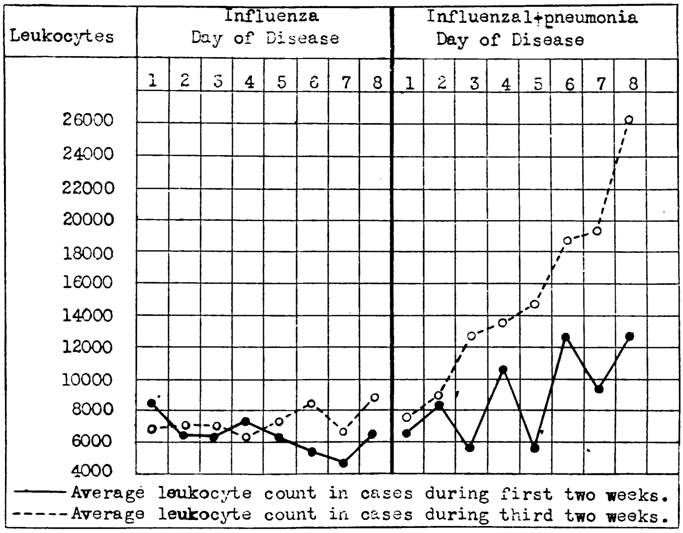

Fig. 2.—Average leukocyte count by days according to time in epidemic waves.

It has been our observation that the most typical cases of influenza and the highest mortality rate occurred at the height of epidemic waves. It was thought worth while, therefore, to group the cases with leukocyte counts according to the time in epidemic waves (figure 2), and to learn whether the average leukocyte count might not be lower in the cases at the height of epidemic waves as compared with that in the cases as the epidemic waves subsided. It was found that each of the four waves of influenza that occurred in Rochester during the fall of 1918 and winter and spring of 1919 ran its course in about six weeks, and that the crest of the wave was reached in about two weeks in each. Each wave was therefore arbitrarily divided into two-week periods for study. The average counts by days of patients developing influenza and influenzal pneumonia during the first two weeks and the third two weeks were determined and traced in figure 2. The average count for the first five days of the influenzal attack in the two groups was about the same; after that the count was decidedly lower in the group contracting the disease during the first two weeks of each wave than during the third two weeks. The average count during influenzal pneumonia was about the same during the first two days and no higher than at the outset of influenza, but after that the rise in leukocytes was decidedly less during the first two weeks than during the third two weeks of each wave. The mortality rate in the first two weeks was definitely higher than in the third two weeks.

In table 1 are given the leukocytic reaction and other findings in guinea-pigs injected with material, first, from patients showing marked leukopenia, and second, from patients showing no reduction or a moderate increase in the leukocyte count. It will be seen that the average leukocyte count in the five patients (table 1, part I) showing leukopenia was 4,340. Two of these died. Four had influenzal bronchopneumonia, and one (case 2,981) showed symptoms of intestinal influenza. All of the 19 animals injected with material from this group of patients showed reduction in the leukocyte count on the day after injection. In some instances this was very marked (P 961, P 981, P 1,069), while in others it was relatively slight. The average diminution in the leukocyte count was 58%. The average reduction 48 hours after injection was 36%. The reduction occurred over a wide range of dosage, 0.1 c c to 1.5 c c. It followed subcutaneous as well as intratracheal injections of green-producing streptococci isolated from the throat, sputum, lungs, blood, and stool. The cultures of streptococci injected were in the first to the fourth generation, and some were derived from single green colonies on blood-agar plates. Injection of a filtrate of a fresh culture of green-producing streptococci also showed reduction in leukocytes (P 996, P 997). This is in accord with the findings following injection of other filtrates (table 2). The mortality in this group of animals was 47%. The reduction in leukocytes twenty-

TABLE 1

Leukocytic Reaction in Guinea-Pigs Following Injection of Material from Influenza and Influenzal Pneumonia

I. Cases Showing Marked Leukopenia

Guinea-Pigs Injected											
				Material Injected			Leukocyte Count				
Number	Leukocyte Count	Result	Number	Dose in c c	Organisms in Glucose Broth Culture or Sputum	Culture Generation	Place	Before Injection	Twenty-Four Hours after Injection	Forty-Eight Hours after Injection	Findings
2,800	2,800	Death	P951	1.5	Hemolytic streptococcus, throat	3	Trachea	26,000	21,000	19,200	Marked increased respiration; subnormal temperature; death sixth day from bronchopneumonia; acute peritonitis apparently secondary to colitis; green streptococci from blood
			P955	1.5	Green streptococcus, throat	2	Trachea	16,000	11,000	17,000	Sick; marked increase in respiration for two days; weight from 450 gm. to 320 gm.; recovered
			P959	0.1	Sputum, green streptococcus	0	Trachea	16,400	6,200	19,000	Fever; increased respiration for three days; recovered
			P961	1.5	Green streptococcus, throat	2	Subcutaneous tissue	10,400	2,400	1,600	Fever; increased respiration for two days; weight from 420 gm. to 320 gm.; death fourth day of bronchopneumonia and hemorrhagic edema; green streptococci from blood, lung, and uterus
			P971	1.5	Green streptococcus, lung	1	Trachea	11,000	9,000	27,000	Fever for two days; recovered
			P973	1.5	Green streptococcus, blood	1	Trachea	17,600	10,600	25,000	Remained well
			P981	1.5	Green streptococcus, blood	2	Trachea	8,000	2,000	6,200	Respirations slightly increased for two days; recovered
			P983	1.5	Green streptococcus, blood	3	Trachea	8,500	4,600	5,400	Increased respiration for a time, then better; death on eighteenth day from marked lesions in stomach and throughout intestinal wall; green-producing streptococci and B. coli from blood, uterus, stomach, and intestinal contents
			P984	1.5	Green streptococcus, blood	2	Trachea	8,800	4,400	9,600	Respirations increased for several days
			P993	1.0	Green streptococcus, blood	3	Subcutaneous tissue	26,000	12,000	9,200	Subnormal temperature; death in 48 hours; emphysema (13 c c), edema and hemorrhage of lungs; subcutaneous cellulitis; countless green streptococci
			P994	1.5	Green streptococcus, blood	2	Trachea	6,800	4,800	15,200	Subnormal temperature; death on seventh day from bronchopneumonia
			P995	1.5	Green streptococcus, blood	2	Trachea	9,600	6,200	14,400	Increased respiration for a few days, then better; death from bronchopneumonia eighteenth day after injection; hemorrhagic pleuritis, myocardial degeneration; countless green streptococci
			P996	1.5	Green streptococcus, blood (filtered)	2	Trachea	11,000	5,000	5,400	Remained well

			P997	1.5	Green streptococcus, blood (filtered)	2	Trachea	15,600	9,800	8,200	Remained well
			P1056	1.5	Green streptococcus, blood	4	Trachea	12,000	6,600	5,000	Increase in temperature and respiration for three days; recovered
2,770	3,800	Death	P870	0.1	Green streptococcus, sputum	1	Trachea	17,000	8,900	5,800	Diffuse coalescing bronchopneumonia; death on seventh day from hemorrhagic pleuritis; green streptococci from blood, lung, and pleura
2,873	6,200	Recovery	P1069	1.5	Green streptococcus, sputum	2	Trachea	11,600	4,200	......	No noteworthy symptoms
2,981	5,400	Recovery	P1174	1.5	Green streptococcus, sputum	2	Trachea	18,000	6,800	9,400	Increased respiration for two days; recovered
3,208	3.500	Recovery	P1261	1.5	Green streptococcus, stool	1	Trachea	11,800	7,000	5,800	Death in 48 hours; diffuse coalescing bronchopneumonia and acute myocardial degeneration; large number of green streptococci from blood, lung, pleural and peritoneal fluid
Average	4 340	....	....	.....	....	.....	....	18,000	7,500	11.570	Total: 19 animals injected; 9 died

II. Cases Showing No Reduction or Moderate Increase in Leukocyte Count

2,616	9,300	Death	P755	3.0	Green streptococcus, lung	0	Peritoneal cavity	15,000	12,000	9,400	Recovered
2,619	7,700	Recovery	P749	0.3	Sputum, green streptococcus	0	Peritoneal cavity	5,600	10,800	6.600	Recovered
			P750	0.3	Sputum, green streptococcus	0	Peritoneal cavity	14,400	4,600	......	Increased respiration; expiratory difficulty; relieved with epinephrin; death 48 hours after injection; emphysema; hemorrhagic edema; bronchopneumonia; serofibrinous peritonitis; green streptococci from blood and peritoneal fluid
2,620	11,100	Recovery	P752	0.3	Sputum, green streptococcus	0	Peritoneal cavity	25,500	20,500	6,800	Recovered
2,621	14,500	Recovery	P751	0.3	Sputum, green streptococcus	0	Peritoneal cavity	6,600	12,750	15,300	Recovered
2,798	12,500	Recovery	P957	1.5	Hemolytic streptococcus	2	Trachea	16.000	19,000	16.500	Increased respiration; death sixth day from hemorrhagic pleuritis; countless numbers of green streptococci in pleural fluid and lung
			P1109	5.0	Hemolytic streptococcus	2	Stomach	12,000	7,400	......	Remained well
2,887	11,000	Death	P1110	2.0	Hemolytic streptococcus	2	Trachea	7,600	6,000	11,[illegible]00	Increased temperature and respiration rate for two days; recovered
3,206	11,300	Recovery	P1258	1.5	Primary culture, sputum, green streptococcus	1	Trachea	11,000	12,000	......	Death in 24 hours from acute hemorrhagic edema and hemorrhagic pleuritis; pleural fluid, lung, and blood showed countless numbers of staphylococci
3,207	13,000	Recovery	P1260	1.5	Primary culture, sputum, green streptococcus	1	Trachea	11,200	10,800	......	Death fourteenth day from bronchopneumonia due to green streptococcus
Average	11 300	....	....	.....	....	.....	....	12,490	11.580	10 960	Total: 10 animals injected; 4 died

four hours after injection was practically the same in the fatal and nonfatal infections, whereas forty-eight hours after injection the leukocyte count was usually lower in the animals that died as a result of the injection than in those that recovered (P 961, P 983, P 870, P 1,261).

Injections were made of material from 8 patients with undoubted influenza but who showed no reduction, or even a slight increase, in leukocytes at the time the animal experiments were performed (table 1, part II). The symptoms of these patients were the same as of those in the former group, although not so severe. The average leukocyte count was 11,300; two of these patients died. Four of the patients had influenza without lung findings, and 4 influenzal pneumonia (1 complicated with empyema) at the time of the animal injections. The leukocyte counts in the 10 animals injected with material from these patients averaged 12,490 the day before injection. Six of the animals showed slight reduction in leukocytes and 4 showed a slight increase twenty-four hours after injection; the total reduction in leukocytes was only 9%. The total average reduction of leukocytes in forty-eight hours after injection was also slight (11%). It is thus evident that injections of material from the patients showing marked leukopenia caused a greater reduction in leukocytes (58%) twenty-four hours after injection than the material (9%) from patients showing little or no reduction.

In table 2 is summarized the incidence of the occurrence of leukopenia, leukocytosis, and no change in the leukocyte count of guinea-pigs following injections of material from patients with influenza (part I), and in a control series with material from sources other than patients with influenza (part II). Injections of sputum, primary cultures from sputum, pure cultures of green-producing streptococci and hemolytic streptococci from patients with influenza were followed by the occurrence of leukopenia in a high percentage of the animals injected and to a lesser degree (33%) following injection of influenza bacilli. Moreover, injection of filtrates from sputum, lung emulsions, and broth cultures likewise was followed by a reduction in leukocytes in 65% of 26 animals injected. The total average of 99 animals in which leukocyte counts were made following injection of material from influenza which showed leukopenia was 57%; leukocytosis, 12%, and no change in the leukocyte count, 29%.

Sharp reductions in leukocyte counts were noted also following injection of staphylococci from the lungs or sputum of patients with influenza. This in some instances was as marked as following the injection of the green-producing streptococcus from the same case. Thus, 2 guinea-pigs were injected intraperitoneally with these strains isolated from single colonies on a blood-agar plate inoculated with the peritoneal fluid of a guinea-pig that died from injection of sputum. The one injected with Staphylococcus aureus showed 9,200 leukocytes before injection; twenty-four hours after injection, 3,200; and forty-eight hours after injection, 6,000. It died of peritonitis and pancreatitis with moderate emphysema of the lungs, and a few small, localized hemorrhages, with a leukocyte count of 3,240, and large numbers of hemolytic staphylococci in the blood and peritoneal fluid. The one injected with the green-producing streptococcus showed 6,000 leukocytes before injection; twenty-four hours after injection, 4,800, and forty-eight hours after injection, 3,600. This animal also died in three

TABLE 2

THE LEUKOCYTIC REACTION IN GUINEA-PIGS INOCULATED WITH MATERIAL FROM PATIENTS WITH INFLUENZA AND FROM OTHER SOURCES

I. Material from Patients with Influenza

Injection	Place of Injection	Animals	Percentage of Animals Showing		
			Leukopenia	Leukocytosis	No Change
Sputum	Trachea	5	80	0	20
Sputum	Peritoneal cavity	5	40	20	40
Primary culture of sputum	Trachea	9	78	11	11
Green-producing streptococci	Trachea	41	46	17	34
Green-producing sterptococci	Peritoneal cavity	4	75	0	25
Hemolytic streptococci	Trachea	6	83	17	0
Influenza bacilli	Trachea	3	33	0	67
Filtrates from lung emulsions and broth cultures	Trachea	26	65	7	27
Total	....	99	57	12	29

II. Material from Sources Other Than Patients with Influenza

Injection	Place of Injection	Animals	Leukopenia	Leukocytosis	No Change
Hemolytic streptococci from a patient with simple nasopharyngitis and leukocytosis	Trachea	3	0	67	33
Sputum and cultures of green streptococci from a patient with simple tracheitis, nasopharyngitis and leukocytosis	Trachea	7	29	57	14
Type pneumococci from patients with lobar pneumonia	Trachea	9	11	22	67
Broth (controls)	Trachea	6	16	17	67
Total	....	25	16	36	48

days with serofibrinous peritonitis, beginning pericarditis, acute pancreatitis, and moderate emphysema of the lungs, showing a few small circumscribed hemorrhages and large numbers of green-producing streptococci in pure culture in the blood, together with a moderate number of staphylococcus aureus in the pericardial and peritoneal fluid. The blood-agar plate of the material injected in each of these animals showed pure cultures of staphylococci and green-producing streptococci, respectively.

The percentages following injection of material from sources other than influenza patients are quite different. In this group the first case was that of a patient with a severe attack of nasopharyngitis and sinusitis, in which the throat was extremely red and the tonsils absent, and in which much mucopurulent material, at first tinged with blood, came from the nose and throat. The attack began with marked chilliness, but no distinct chill, and was followed by fever for a number of days and a leukocytosis of 14,000 on the first day. The patient recovered without developing symptoms of infection of the trachea or bronchi. The hemolytic streptococcus in the second culture generation isolated from the throat produced, on intratracheal injection, leukocytosis in 67% of the animals. The patient, it should be noted, developed no symptoms suggesting infection of the trachea or bronchi and the streptococcus caused no deaths on intratracheal application. This finding is in sharp contrast with the results of injection of the hemolytic streptococci from influenza in which leukopenia occurred in 83% and leukocytosis in only 17% of the animals injected, and in which death from hemorrhagic edema, hemorrhagic pleuritis, or bronchopneumonia occurred in a high percentage of animals injected (figure 1).

The results in the second case are also in sharp contrast to those obtained with material from patients with influenza. In this case the patient awoke in the morning with marked soreness in the upper part of the chest and a painful cough by which thick, mucopurulent material was raised. The day following, the cough and soreness in the chest continued and typical symptoms and signs of a nasopharyngitis had developed, with sneezing and abundant mucous discharge associated with redness of the mucous membranes of the nose and throat. There was little general aching, slight fever, and a leukocytosis of 13,500 on the second day. Recovery was practically complete on the fourth day. Intratracheal injections of the sputum and cultures of the green-producing streptococcus in the primary culture from the nasopharynx, and the same organism in the third culture generation isolated from the sputum on the second day was followed by leukocytosis in 57% of the animals. There was leukopenia in 29% and no change in leukocytes in 14%. None of the animals died of acute hemorrhagic pulmonary edema or hemorrhagic pleuritis. Three developed mucopurulent discharge from the nose in which the streptococcus injected was present in large numbers.

The intratracheal application of type pneumococci isolated originally from lobar pneumonia and proved virulent on intraperitoneal injection just prior to intratracheal injection was followed by leukocytosis in 22%, leukopenia in 11%, and no change in the leukocyte count in 67% of the animals injected. The relatively few instances of leukopenia following injection of the cultures from sources other than influenza usually occurred in animals that succumbed from overwhelming infection. Most of the intratracheal injections consisted of broth cultures of the different bacteria. Control injections of broth in the same dosage were followed by temporary leukopenia in 16%, leukocytosis in 17%, and no change in 67% of the animals injected. In the experiments in which filtrates of the cultures were injected the broth and culture filtrates were of the same batch. The occurrence of leukopenia in 65% of the animals injected with filtrates in contrast to 16% of those injected with broth represents roughly the "leukotoxin" formed by the growth of the streptococci in the broth. Filtrates of pneumonic lungs also possessed this power to a marked degree. In most instances 0.2% glucose-broth cultures and filtrates of cultures in this medium were injected. The broth cultures usually developed an acidity to phenolphthalein of from 1.5 to 2.5%. A series of experiments was made in which parallel intratracheal injections of 0.5 c c for each 100 gm. of body weight of cultures of acid reaction were given, and the same cultures injected after neutralization with sodium hydroxid. There was no noteworthy difference in the two sets of animals, in the leukocytic reaction, in the immediate respiratory symptoms, or in the late results from infection. Moreover, the "leukotoxin" or the property in broth culture filtrates which causes leukopenia and the symptoms of anaphylactic shock is not destroyed by heating to 60 C. for thirty minutes. (See experiments with filtrates.)

The degree of leukopenia following intratracheal injection of these cultures was found to be roughly proportional to the severity of respiratory embarrassment in the animals. In the case of extremely toxic cultures, a sharp reduction has been noted within a few hours after intratracheal application, and even after intranasal insufflation. In some instances the reduction in the leukocyte count did not occur for several days and then with the occurrence of death, with voluminous hemorrhagic and edematous lungs showing few leukocytes in the alveolar exudate, due to streptococci, a sharp drop in leukocytes was sometimes noted. On the other hand, in the animals that died late and that showed leukocytosis, the lungs were not so voluminous, the areas of consolidation were more firm, less moist on the cut surface, peribronchial in location, and the alveolar exudate was rich in leukocytes. These animals also often showed purulent bronchitis and tracheitis.

The leukopenia following direct inoculation of sputum was no greater and occurred no oftener than following injection of pure cultures of the streptococci. The latter were usually derived from single colonies and were in the first to the eleventh culture generation. The animals that succumbed following injection of sputum nearly always showed green-producing streptococci, and injection of pure cultures of these produced marked leukopenia often to a greater degree than those in the first animal passage.

This finding indicates that the reduction in leukocytes following injection of the material from patients with influenza may not be due to an unknown virus, but to peculiar properties of the streptococci or other bacteria at hand in this disease. These streptococci have the power by growth in vitro and in vivo to produce a soluble filterable substance. The filtrates from broth cultures and from influenzal lungs and sputum when applied intratracheally in guinea-pigs have the power, among other properties, to cause sharp reduction in leukocytes. The reduction in leukocytes has occurred with regularity only in the animals injected with influenza strains and not following injection of streptococci in like dosage from similar conditions showing leukocytosis, or type pneumococci. The average degree of leukopenia in the animals was roughly proportional to that found in the patients from whom the strains were isolated, and the leukocyte curves in fatal and nonfatal infections in the animals correspond roughly to those noted in the spontaneous disease in man. The conclusion seems warranted, therefore, that the leukopenia in influenzal infection in man may be due to peculiar properties of the bacteria which are now generally regarded as secondary invaders and not to an unknown virus. Moreover, according to our findings, a persistent, marked leukopenia, or an increasing leukopenia in influenza predisposes to influenzal pneumonia and in the latter indicates a bad prognosis.